DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

A METHOD FOR ORIENTING AND MOUNTING MICROSCOPICAL OBJECTS IN GLYCERINE

BY

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The object of this paper is to describe a method of mounting desmids or similar microscopical objects in glycerine, so that they may not only be drawn in different positions with the camera lucida, but may form the basis of an herbarium of mounts which may be regarded as permanent, since the oldest preparations made by this method, now about twenty-five years of age, show no signs of deterioration. The account herewith presented is offered for the purpose of enabling others to utilize it, as well as to answer certain inquiries that have been made in regard to the subject. In its details the procedure presents no novel features. Its principles are those used in the laboratory of Professor Thaxter for mounting the lower fungi and other of the more delicate Thallophytes in glycerine, or glycerine and eosin, and sealing with King's Cement.[1] This method is especially well adapted for mounting desmids for study from the point of view of the systematist; since, in the vast majority of species only the well developed empty cells and semi-cells are useful for this purpose. Those species of Cosmarium or of Spirotaenia, for example, the cell-contents of which have to be studied, require more exact fixation methods, with which this paper is not concerned.

In mounting a sufficiently large object, no "finding ring" is needed. But for marking the position of a small or large series of minute objects, it is often essential. A ring of Brunswick Black has been found most serviceable for this purpose, and should be prepared months in advance, so as to become well seasoned. It may be placed centrally on the slide by means of the turntable, and need not be larger than the field of the low power.

THE MATERIALS AND THE METHOD OF THEIR USE

Since the procedure here described involves the use of a weak glycerine jelly as a means of orientation, it is necessary, in order to avoid the diffi-

[1] King's Cement was an invention of the Rev. J. D. King of Cottage City, Massachusetts who did not publish the formula or method of preparation. There is an antiquated recipe published on page 235 in Rev. A. B. Hervey's translation of Behren's "Guide to the Microscope in Botany" (S. E. Cassino Co., Boston 1885). Dr. Hervey assures me that it is genuine, as he received it directly from King. If the more modern form of this cement cannot be obtained from a dealer, it can be bought of its present maker, Professor R. E. Schuh, Howard University, Washington, D. C.

culties inevitably associated with the process in warm weather, to perform the manipulations indicated in the cooler part of the year. After the objects are oriented, the weak jelly may be satisfactorily set by placing the slide-box containing the preparations outside a window where it will be chilled. The drawing can be done in the warm part of the year, when the light is also best. This weak jelly is made from any good clear glycerine jelly, such as that prepared by Kaiser's formula. A few drops of melted jelly in a small vial is reduced by the addition of boiled or distilled water, until the mixture will just set at the ordinary temperature of the room. It should be perfectly limpid. The cork may be furnished with a dropper, by pushing into its lower end a piece of platinum wire of such length that a small loop at the lower end nearly touches the bottom of the vial. The vial should be kept well corked.

Objects may be lifted, transferred and oriented by means of a fine needle such as "No. 12 Sharps." The needle is pushed eye first into the end of a large match of straight grained wood, until a quarter of an inch, or less, of the pointed end, which is thus as rigid as possible, remains projecting. A smaller instrument may be made by the addition of a proper bristle, for which purpose a carefully selected whisker of a cat or dog answers admirably; since it combines stiffness and elasticity with an extremely delicate point. This bristle should be cemented to the mounted needle, and bound in place by means of a long human hair, or a fine waxed silk thread, in such a position that the point of the bristle projects slightly. It is important in order to obtain the necessary rigidity that the free portion of the bristle should be as short as possible consistently with convenient use.

The half inch circular cover glass is best adapted for general use, and in mounts of this nature, it is necessary to employ a shallow cell. This may be readily made by supporting the cover at one side by either a somewhat compressible, or an entirely rigid support. The latter results in a better mount mechanically, the former is easier to work with. The more flexible support, consists in fibers of blue blotting paper completely picked and teased out and then felted together again into a ball by means of the forceps. This material has the advantage of compressibility, which permits one to vary a little the amount of glycerine used for the mounting medium. It has a certain disadvantage, however, from the fact that, as it is not a rigid support, care must be taken that a mass of sufficient size is used, to prevent the cover from touching the object, after the cement has dried and contracted. A rigid cover glass support may be made by selecting a very thin cover glass and placing it in an elongated folded paper. If this is pressed against the edge of the table and drawn back and forth, the glass within will be finely comminuted. A minute fragment of this may then be

used to support the cover. One must learn by experience the approximate amount of glycerine needed to fill the space beneath the cover.

The cement may be kept in an ounce, or half-ounce, wide mouthed bottle. Into the bottom of the cork a match, bearing the ringing brush is inserted; the point of the brush nearly touching the bottom of the bottle. The ringing brush itself should be the smallest obtainable with not many but rather long hairs. A match may be pushed into the quill and firmly bound with silk, and the whole fitted to the cork as mentioned.

For a mounting medium it is best to use only chemically pure glycerine, filtered if necessary. It may be kept in a vial to the cork of which is fitted a platinum loop, or a properly selected mourning pin, by means of which the glycerine may be conveniently applied.

Manipulations

The details of the procedure for mounting large and small objects are the same, but the smaller species are obviously the more difficult. A slide is prepared for the reception of the objects by placing a minute smear, or streak of the weak jelly, within the ring previously prepared; or if desirable a series of minute drops may be used. It is convenient to employ an ordinary dissecting stand, with a x12 aplanatic triplet, on which the slide thus prepared is left, with the focus and light exactly regulated. The material to be mounted having been spread out in glycerine on a slide, the particular individual desired is selected under the compound microscope, and pushed about with the needle beyond the edge of the glycerine until freed from glycerine and all extraneous matter. In this condition it will readily stick to the needle, and can thus be lifted from the slide, transferred to the surface of the weak jelly, and there left until all the individuals desired for this mount have been transferred. The weak jelly should then be liquefied by breathing gently upon it, and the slide at once placed under the low power of the compound microscope. It will then be found that, with a little practice, the objects can be easily and systematically arranged with the needle. They may be set up in lines, or curves, in whatever order may prove most convenient. Should the jelly harden too rapidly, it may again be liquefied as above described. In a short time, one acquires skill in setting up objects, such as desmid semi-cells, in different positions under the compound microscope, without disturbing those already in position. For drawing care should be taken so to set up a symmetrical object that its vertical axis coincides with the optical axis, or nearly so. The horizontal axes may be pointed in any direction, by merely revolving the slide on the stage. After placing and orienting the specimens, the slide may be put away upside down in a slide box, until two or three more are brought to the same state of preparation. In this con-

dition they may be left outside a window all night in order to harden the jelly.

When the preparation is ready to mount and seal, the support of blotting paper, or cover chip, already mentioned, should be placed near the edge where it will be just included by the cover as it is lowered. A clean drop of glycerine should then be placed on the object, sufficient to fill the cell as exactly as possible. If blotter shreds are used, and the glycerine does not quite fill up the cell after the cover is placed in position, cautious pressure over them with the point of the needle will spread the glycerine, and fill the cavity completely. It may then be carefully sealed. If more glycerine must be added to fill a cell, a small drop should be placed at a short distance from the edge of the cover, and a narrow streak of it drawn with the needle to the edge of the cover; so that a little will flow beneath it. After repeating this process with the needle till the cell is completely filled, the surplus glycerine must be carefully wiped off.

The complete removal, before sealing, of this surplus is absolutely essential for the preparation of a permanent mount; since this is the only way to prevent subsequent leakage. A very little cement under the edge within the mount serves to make it stronger. In order to remove all trace of glycerine, it must be very carefully wiped off by means of an old, much washed handkerchief, folded over the end of the forefinger in such a manner as to form a point, which is moistened with alcohol. This can safely be pushed up till it touches the edge of the cover, and then repeatedly renewed and worked around it until all glycerine is removed. In case a still larger surplus of glycerine must be removed, it is convenient to use small strips of blotter folded ∧ shape. One end is moistened with alcohol and pushed up against the edge of the cover. Several pieces about the cover edge absorbing simultaneously will gradually remove most of the excess, after which, the slide must be very carefully cleaned with the handkerchief, as before mentioned, and the mount at once sealed.

When there is only one specimen, and different aspects of it must be drawn, the preparation is demounted after the first figure is made, and before sealing the mount. The cover is lifted off, the glycerine drained away, the jelly again liquefied as before, and the object transferred to another prepared slide, remounted in a new position and drawn again. The last remount, showing the object in its most characteristic position, may be sealed.

To seal a perfectly cleaned mount, it may be held in the unsupported left hand, while with the right, a light ring of cement is applied, after resting the right hand on the left. With a full brush, a drop of cement is then started on the edge of the cover, the hands being held as before, and led around the circle by means of the brush in such a manner that the

cement ring is partly on the cover and partly on the slide. The first ring thus helps to keep the second under better control. Additional rings may be applied on the turntable, when the sealing rings are hard. It has been found convenient to finish with two coats of Brunswick Black. This is soluble in turpentine, and offers more resistance to the solvent action of alcohol used to clean immersion oil from the cover.

In glycerine mounts of most objects, collapsed specimens regain their turgescence; and air bubbles, if present, disappear in a few days. Since these mounts are delicate, they must be handled with care, and always kept horizontal. Any necessary modifications of this method may be made in order to mount other microscopical objects needing orientation for camera drawing, and thus make it possible to obtain more accurate figures. But the fact that mounts thus sealed have shown no signs of leakage for so many years, indicates that the correct principles have been applied at this critical point, and should not be lightly changed.

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